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Manganese in drinking water

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Förord

Föreliggande utvärdering angående hälsoeffekter av mangan i dricksvatten har utförts på initiativ av Socialstyrelsen och på uppdrag av Naturvårdsverket, under programområde Hälsorelaterad miljöövervakning. Texten är avsedd som kunskapsunderlag för Socialstyrelsens arbete med mangan i dricksvatten från enskilda brunnar. Texten är även avsedd som informationsmaterial till övriga myndigheter och forskarsamhället om mangans förekomst i dricksvatten och om dess eventuella hälsoeffekter.

Hos vuxna förekommer en noggrann reglering av mangans upptag från mat och dryck. Denna är dock ej helt utvecklad hos spädbarn. Rapporten har därför främst fokuserat på mangans hälsoeffekter på spädbarn då dessa utgör den största riskgruppen. Det är främst spädbarn som ej ammas som riskerar att exponeras för höga manganhalter från bröstmjölksersättning och dricksvatten. Arbetet är baserat på tillgänglig vetenskaplig litteratur samt på det vetenskapliga underlaget till nuvarande riktvärden för mangan i dricksvatten.

Arbetet har utförts vid Institutet för Miljömedicin (IMM), Karolinska Institutet under vintern/våren 2006/2007. Rapporten har granskats och kommenterats av Marianne Löwenhielm och Ing-Marie Olsson vid Enheten för hälsoskydd på Socialstyrelsen. Uppgifter om manganhalter i svenska brunnar har vänligen upplåtits av SGU, där diskussioner främst förts med Göran Risberg vid Enheten för hydrogeologi.

Sammanfattning och slutsatser

Mangan är en metall som förekommer naturligt i vår miljö. Det är ett ämne som har många användningsområden, främst som en komponent i stål, men det används även i färgämnen, rengöringsmedel, svampmedel, blekmedel, tändstickor, fyrverkerier, batterier, som näringstillskott till växter, djur och människa, inom röntgentekniken och som blyersättningsmedel i bensin. Det största tillskottet till vår miljö är dock naturligt, dvs det kommer från berggrunden. Människor får i sig mangan främst genom födan, där cirka 20% av vuxnas dagliga intag beräknas komma från dricksvatten. För spädbarn som främst får bröstmjölksersättning kan dock dricksvatten utgöra en betydande källa. Dessutom innehåller bröstmjölksersättning i sig ofta avsevärt högre halter än de som återfinns i bröstmjölk.

Mangan är ett essentiellt ämne som kroppen behöver för en mängd funktioner. Det är därför viktigt för fostrets normala utveckling. Dock har studier visat att högt intag hos framförallt barn, men även hos äldre, kan påverka nervsystemet, vilket visar sig framförallt genom störningar på beteendet. Samband mellan manganintag och neurotoxicitet har påvisats för olika biomarkörer (manganhalter i blod, hår och konsumerat vatten), olika kognitiva test och olika exponeringskällor. Även om ingen av studierna är helt övertygande med avseende på mangans toxiska effekter på barn, indikerar antalet studier som ändå funnit någon form av samband att barn är mer känsliga för mangans negativa effekter än vuxna. Fler studier behövs dock för att bekräfta dessa samband.

Socialstyrelsens allmänna råd för mangan i dricksvatten från enskilda brunnar (300 µg/L) skyddar sannolikt vuxna och ungdomar från ohälsosam exponering. Det är även troligt att riktvärdet skyddar foster samt ammande spädbarn, eftersom transporten av mangan via modersmjölk och placenta verkar vara begränsad. Riktvärdet är under den lägsta halten där negativa effekter har rapporterats i samband med neurotoxiska effekter hos en grupp människor över 50 års ålder (2000 µg/L). Det är dock förhastat att dra slutsatser från endast en studie, vilket visar på behovet av vidare forskning kring äldres exponering för mangan och eventuella samband med Parkinsons-liknande symptom. Det finns även behov av ytterligare forskning på transporten mellan mor och foster.

Ett flertal studier har funnit samband mellan högt manganintag och effekter på barns beteende. Det är i nuläget oklart om ett högt manganintag påverkar både yngre och äldre barn, eller om symptom som påvisats hos äldre barn är effekter från manganintag vid en yngre ålder. Det nuvarande riktvärdet på 300 µg/L är dock lågt nog för att skydda barn över ett år från negativa effekter, då det endast utgör 15% av ett barns dagliga manganintag (~2 mg/dag) vid en normal vattenkonsumption (ca 1 L/dag).

Den största riskgruppen för överexponering av mangan utgörs av spädbarn som får bröstmjölksersättning. De flesta bröstmjölksersättningar innehåller betydande mängder mangan redan i pulverform, motsvarande 400 µg/L i beredd form. Det är därför viktigt att det vatten som mjölkpulvret blandas ut med har en låg manganhalt. I nuläget överskrids ofta den högsta tillåtna manganhalten för bröstmjölksersättning, 650 µg/L, när vanligt mjölkersättningspulver blandas med vatten innehållandes 300 µg Mn/L. Vi vill dock framhålla att det finns en del frågetecken även kring det vetenskapliga underlaget till den högsta tillåtna halten av mangan i bröstmjölksersättning. Det rekommenderas därför att både vatten och mjölkersättning som avses för spädbarn innehåller så låga manganhalter som möjligt för att förhindra att den färdiga produkten innehåller halter som väsentligen överstiger de som förekommer normalt i human bröstmjölk.

Socialstyrelsens allmänna råd om en högsta manganhalt i vatten vid 300 µg/L är inte juridiskt bindande. Det är därför upp till varje hushåll med enskilt vatten att ansvara för sin egen vattenkvalitet. För att förhindra att spädbarn exponeras för alltför höga manganhalter genom dricksvatten bör föräldrar informeras om vikten av ett lågt manganintag för att minimera eventuella risker för barnets hälsa. Enligt den internationella koden för marknadsföring av bröstmjölksersättning får information om mjölkersättningsprodukter endast ges av ”personal som är knuten till hälso- och sjukvården och får endast anordnas för föräldrar vilkas barn är i behov av sådana livsmedel” (SOSFS 1983:21). Detta bör därför även vara en passande och effektiv informationskanal till föräldrar om vikten av låga manganhalter i spädbarns kost, samt om hur överexponering av mangan kan undvikas.

Om kranvattnet innehåller förhöjda manganhalter kan flaskvatten användas för att blanda till mjölkersättning till spädbarn. I Sverige finns det idag fyra typer av buteljerat vatten. Manganhalterna i naturligt mineralvatten och källvatten får

ej överstiga 500 µg/L, medan det för förpackat dricksvatten och bordsvatten ställs samma krav som på drickvatten från kommunala vattenverk; 50 µg/L. Dessa två vatten med lägre manganhalt kan därmed användas istället för kranvatten, om vattnet från den enskilda brunnen ger förhöjda manganhalter. Mangan kan även filtreras bort från kranvatten.

Sammanfattningsvis kan sägas att det fortfarande föreligger en hel del osäkerheter angående vilka lägsta manganintag som kan innebära negativa hälsoeffekter hos spädbarn och barn, och även hos äldre och hos foster. Vidare forskning behövs för att förstå sambanden mellan manganintag och barns hälsa, och för att möjliggöra en förbättrad hälsoriskbedömning. Det bör utredas hur biotillgängligheten av mangan i bröstmjölk skiljer sig från den i mjölkersättning och vatten, samt hur upptag och retention varierar med ålder. Det behövs även en tillförlitlig biomarkör för att kunna utvärdera dos-respons förhållanden för mangan.

Karolinska Institutet

CONTENTS

1 Manganese in the environment

Manganese (Mn) is an essential trace element for all living organisms. It plays an important role in various parts of metabolism in humans and animals as well as in microorganisms and plants (Brady and Weil, 1996; Klaassen, 1996). Its chemical and physical properties are very similar to iron, but manganese is harder and more brittle (NPI, 2004).

Rock weathering and wind erosion cause release of manganese into the surrounding soil, water and atmosphere. This natural re-distribution of manganese is generally more important for the manganese concentrations in soil, plants, water and air than manganese from anthropogenic sources (Reimann et al., 1998).

1.1 Anthropogenic uses

Manganese is the fourth most used element in terms of tonnage, with around 34 million tons of ore being mined annually. It is an important component of steel where 90% of the annual manganese consumption goes into steel and as an alloying element (IMNI, 2007). There are however, a variety of uses for both inorganic and inorganic forms of manganese.

1.1.1 Inorganic manganese

Inorganic manganese and its compounds are widely used in the manufacture of products, ranging from disinfectants and health foods to plant fertilizers and batteries. While manganese oxides and carbonates are used in textile printing, glass and ceramics colouring, potassium permanganate is used as a decolouring agent. Permanganates and manganese chlorides are also used as disinfectants in survival kits, to be used for wounds as well as for disinfecting vegetables. They are used in waste water treatment, metal cleaning, as an anti-algal agent, in bleaching, treatment of ulcers and fungal infections, to remove Fe and H_2S from well water, as a fruit and flower preservative, to purify natural gas as well as in cocaine production.

Many forms of manganese are used in feed supplements for animals and as a food additive and dietary supplement for humans, as well as in crop fertilizers.

Inorganic manganese compounds are also used in the manufacture of matches, fireworks, dry-cell batteries, electrical coils, welding rods and can be mixed with formalin to produce teargas (NPI, 2004).

1.1.2 Organic manganese

Organic forms of manganese are used as a contrast agent in magnetic radiation imaging (MRI), as fungicides (Maneb and Mancozeb) and as an anti-knock agent (MMT). The contrast agent Teslascan™ contains manganese in the form of Mangafodipir trisodium, MnDPDP and is used in MRI of the liver and pancreas (Fass, 2002). The fungicide Maneb is currently banned in Sweden, but there were previously 19 different products containing Maneb that were used primarily as a fungicide for potatoes. Most Maneb preparations were banned during the 70's, with the last one being banned in 1994. Mancozeb is currently still in use in Sweden, and is present in four fungicide products. Eight more products were previously used but were banned during the 90's. There are current restrictions on the use of Mancozeb, where it must not be used on edible parts of the plant and its latest date of application must be at least 30 days prior to harvest (Kemi, 2007).

The anti-knock agent methylcyclopentadienyl manganese tricarbonyl (MMT) contains about 24.4% manganese by weight and the addition to gasoline results in 18 mg Mn/L fuel. When MMT is combusted in the car engine, inorganic manganese particles are produced and released into the surrounding air. These particles have a mean aerodynamic diameter of 0.5-1.0 μm (Ross et al., 2000) and primarily contain phosphate and sulfate forms of manganese, although divalent manganese oxides are also discharged (Dorman et al., 2006). According to Afton Chemicals, a major producer of MMT, 150 refiners in 45 countries throughout the world add MMT to fuel (Afton Chemical, 2007).

1.2 Natural occurrence

1.2.1 Bedrock

Manganese is an abundant element in the earth's crust. It comprises about 0.1% of the crust (Schiele, 1991) and occurs in various primary rocks, very often together with iron (Gounot, 1994; BGS & WaterAid, 2003). It is thus a

naturally occurring element. It is enriched in mafic and ultramafic rocks (1200 mg/kg) while granites show lower values (400 mg/kg). Shales and grey-wackes as well as limestone can show quite notable manganese-concentrations (700-850 mg/kg) (Reimann, et al., 1998; 2003).

Manganese does not exist naturally as a base metal, but is included in more than 100 minerals, including sulfides, oxides, carbonates, silicates, phosphates and borates (WHO, 2004a). The most important manganese mineral is native manganese dioxide, also known as pyrolusite. Its most common oxidation state is Mn^{2+}, but it also occurs as Mn^{3+} and Mn^{4+}. Manganese in its divalent oxidation state is known to replace the sites of some other divalent cations in silicates and oxides, such as Fe^{2+} and Mg^{2+}. Many manganese-containing minerals weather relatively easily resulting in a release from these rocks to the surrounding environment (Reimann et al., 2003).

1.2.2 Soil

Manganese is present in soil as a result of mineral weathering and atmospheric deposition, originating from both natural and anthropogenic sources. There are three possible oxidation states of manganese in soil; Mn^{2+}, Mn^{3+} and Mn^{4+}. The divalent ion is the only form that is stable in soil solution, while Mn^{3+} and Mn^{4+} are only stable in the solid phase of soil (McBride, 1994).

Manganese mobility in soil is extremely sensitive to soil conditions such as acidity, wetness, organic matter content, biological activity etc. The solubility of soil manganese is thus controlled by redox potential and soil pH, where low pH or low redox potential favour the reduction of insoluble manganese oxides resulting in increased manganese solubility. At soil pH above 6, manganese bonds with organic matter, oxides and silicates whereby its solubility decreases. Manganese availability and solubility is thus generally low at high pH and high organic matter content, and high in acid soils with low organic matter content. However, highly alkaline soils at pH above 8 can release enough manganese to produce plant toxicity. Solubility is also high at pH above 6 in anaerobic conditions, as well as in aerobic conditions at pH below 5.5. Since manganese is rapidly mobilized and re-precipitated, there is seldom an association between total soil manganese concentrations and manganese content in the parent material. This sensitivity also results in large fluctuations

of manganese in soil over time (McBride, 1994; Kabata-Pendias & Pendias, 2001).

Worldwide, soil manganese ranges from 80 to 1300 mg/kg with a median of 530 mg/kg (McBride, 1994). The median manganese concentration in agricultural soils of Northern Europe is reported at around 400 mg/kg, while manganese in Swedish agricultural soils is reported at slightly higher concentrations around 550 mg/kg. Finnish morain soils reportedly hold 500 mg/kg (Reimann et al., 2003). Studies on urban soils have found median manganese concentration ranging from just over 130 mg/kg in New Orleans (Mielke et al., 2004) to 470 mg/kg in Seville of Spain (Madrid et al., 2002). In Sweden, the manganese concentration of Stockholm's urban soil was 325 mg/kg (Berglund et al., 1994) and in Falun 140 mg/kg (Sandberg, 1995). In the urban area of Uppsala, the top five cm held just below 500 mg/kg while almost 600 mg/kg was found in the 10-20 cm layer (Ljung et al., 2006). The reported urban concentrations are likely more dependent on geogenic influence rather than anthropogenic input.

1.2.3 Air

Manganese is found in low levels in air originating from both natural and anthropogenic manganese emissions. The natural sources include volcanic emissions, soil and dust erosion as well as a re-suspension of eroded dust (Dorman et al., 2006). Anthropogenic sources of atmospheric manganese include industrial activities, such as iron and steel production plants, power plants, coke ovens, battery production and welding (Dorman et al., 2006). A source that has received much attention lately is the petroleum additive MMT.

Studies of the addition of MMT to air manganese concentrations have been conducted in Canada, where MMT has been used since 1976. The average total respirable outdoor manganese concentration was 25 ng Mn/m^3 which was significantly higher than in the corresponding rural area (5 ng/m^3) (Bolte et al., 2004). Studies conducted in Toronto found that the mean concentration of respirable manganese was 13 ng Mn/m^3 (Pellizzari et al., 2001). The same authors also evaluated manganese concentrations in Indianapolis, a city where MMT is not added to gasoline, and found that both occupational and non-occupational exposures to manganese were on average 4 ng/m^3 lower than in

Toronto. The significance of this difference has not been established and it is unknown whether the difference can be attributed to MMT.

According to Barceloux (1999), the background air concentration of manganese in urban and rural areas without point emission sources range between 10-70 ng/m^3. According to Saric and Piasek (2000), typical urban air concentrations of manganese are between 10-30 ng Mn/m^3. In areas near industrial sources, the level of manganese in the ambient air range from 220-300 ng/m^3. Environmental exposure to inhaled manganese contributes only a small fraction (<0.1%) of a non-occupationally exposed person's total manganese intake (Dorman et al., 2006).

1.2.4 Water

As a result of weathered and solubilized manganese from soil and bedrock, manganese occurs naturally in both surface and ground waters. In addition, manganese is deposited into waters from human activities.

The manganese concentration in water is primarily controlled by pH and redox conditions, where solubility increases under acidic as well as under anaerobic conditions. In neutral conditions, the redox condition is a stronger determinant for manganese mobility than pH. The concentration of manganese under aerobic conditions typical of shallow aquifers and surface water, is generally low and found below detection limits. The reason is that in aerobic conditions, manganese is found in its stable oxidized form, MnO_2, which is highly insoluble.

As water infiltrates downwards through soils and aquifers, the soil environment becomes more anaerobic and more reducing. The reduction reactions follow a sequence in which oxygen is removed first, followed by nitrate and manganese. Progressively more reducing conditions lead to the reduction of iron followed by sulphate. In these anaerobic conditions, manganese is released from minerals and reduced to its more soluble form, Mn^{2+}. This form is the most soluble in most waters. Much higher manganese concentrations are therefore commonly found in anaerobic ground waters than in aerobic surface or shallow waters (BGS & WaterAid, 2003).

Deep wells also tend to have higher manganese concentrations due to the increasingly reducing conditions with well depth, which increases solubility. Because anaerobic conditions are common in deeper aquifers, the problem of elevated manganese concentrations in groundwater is relatively common, although concentrations vary widely. Several areas worldwide have water manganese concentrations above what is considered safe for consumption (WHO, 2004b; BGS & WaterAid, 2003). However, under strongly reducing conditions and in the presence of dissolved sulphide, manganese can be rendered immobile due to the formation of insoluble manganese sulphide (MnS). This is usually only important at pH above 8.

Presence of bacteria and organic matter in water can also affect manganese mobility. In waters rich in organic matter, manganese can form complexes with organic acids which increase manganese mobility. The mobility can be decreased by some types of bacteria, which can gain energy by oxidation of soluble manganese in waters with high manganese concentrations, and can also accelerate the oxidation process. These bacteria produce surface slimes and may exacerbate staining problems (BGS & WaterAid, 2003).

In seawater, the manganese concentration ranges between 0.4 to 10 µg/L with a mean of ~2 µg/L (WHO, 2004b; Reimann et al., 1998). The manganese levels in fresh water are usually higher, ranging between 1 and 200 µg/L (Barceloux, 1999). The world median manganese concentration in stream water is 4 µg/L. In Canada, stream water concentrations range from <0.1 to 250 µg/L, while the median in Finnish stream water is 30 µg/L, ranging from ~1 to 200 µg/L. In Norwegian lake waters, manganese concentrations have been measured between <0.2 and 330 µg/L, with a median value of 3 µg/L. In Swedish lake waters, the average manganese concentration was reported at 36 ug/L, with a range from 0.2 to 550 µg/L. Figure 1 shows the average manganese concentrations in lakes of different regions of Sweden. In Norway, the median groundwater manganese concentration is reported at 7.5 µg/L, ranging between 0.1 and almost 1,000 µg/L (Reimann et al., 1998). In Sweden, data on groundwater from dug wells providing drinking water report median manganese concentrations at 50 µg/L, with a similar range as in Norway.

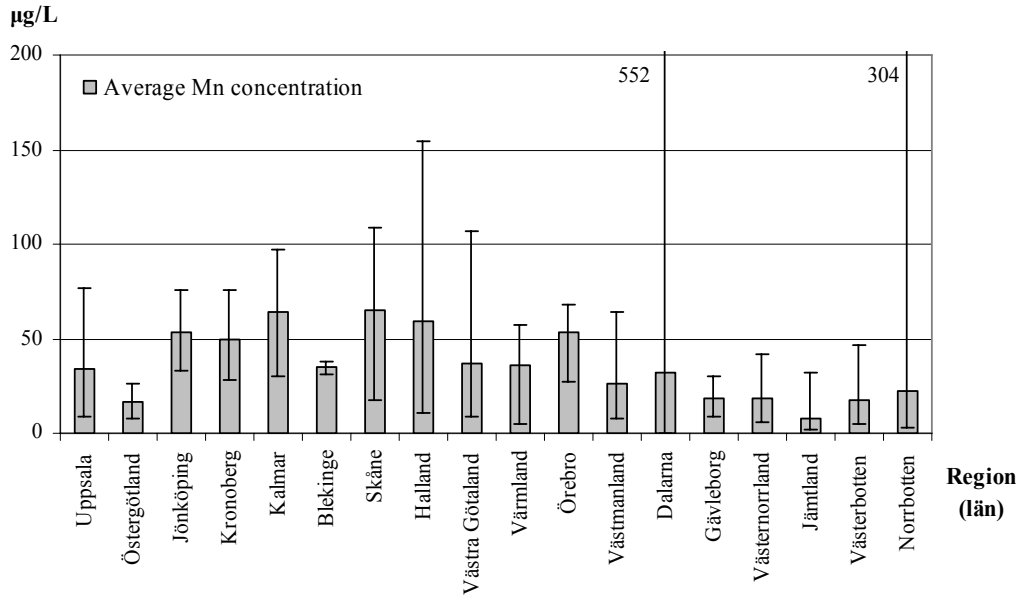

Figure 1. Manganese concentrations of lakes in different regions of Sweden, with error bars showing min and max values (SLU, 2005).

1.3 Manganese in Swedish waters

The increased acidification of Swedish soils has rendered manganese in nutrient-poor soils more mobile, resulting in addition to and transport with ground water to aquifers and lakes (Knutsson & Morfeldt, 1995). Concurrently, drainage of farm lands and the natural land rise cause oxidation of anaerobic sulfide rich soils to acid sulfate soils. As manganese and other metals are mobilized in acid conditions, they are released from soil minerals into soil waters. Dissolved manganese is then easily transported with soil water downward to the ground water through cracks in clayey soil. These effects of drainage on acid soils have been observed in clayey soils along the coasts of Norrland, in parts of Mälardalen and in the south of Sweden (Sohlenius & Öhborn, 2002).

Approximately 1.2 million people in Sweden, about 15% of the population, retrieve their household water from private wells. In addition, another million

people depend on private wells for water supply in their temporary homes, such as summer houses. In total, approximately 400,000 wells supply water to permanent households and just as many to temporary households. About half of these wells are drilled (Socialstyrelsen, 2001). There are no legally binding regulations on water quality for private wells, but a guideline value is set at 300 µg/L in order to avoid the risk of staining on clothes and dishes. In addition to staining problems, elevated manganese concentrations in drinking water also affect the taste and odor of the water (Socialstyrelsen, 2003; Knutsson & Morfeldt, 1995). Naturally elevated manganese and iron concentrations in water are the most common quality problems of Swedish drinking waters (Knutsson & Morfeldt, 1995).

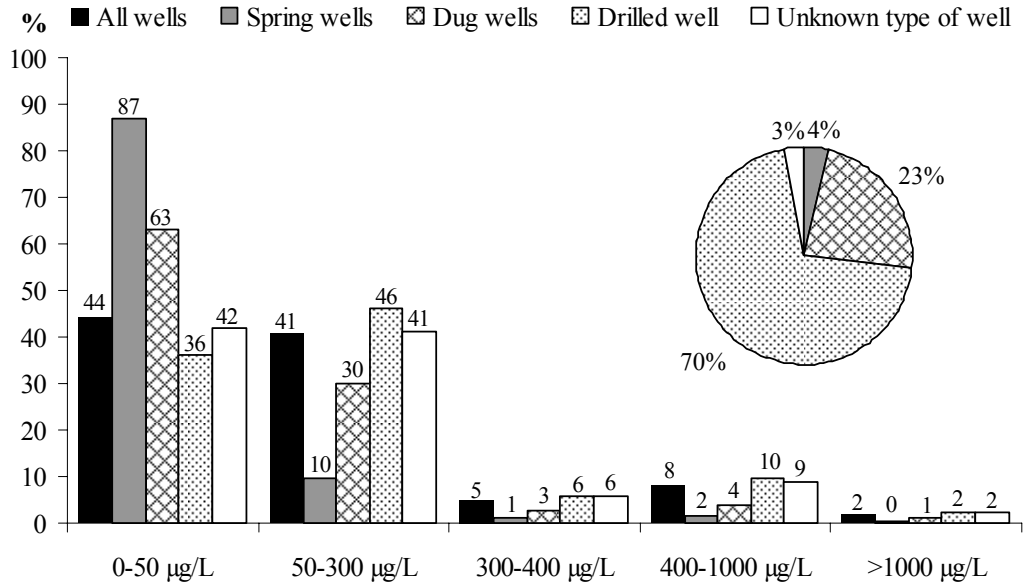

Figure 2. Distribution of manganese concentrations in 18,713 sampled wells in Sweden (SGU, unpublished data).

According to data provided by the Geological Survey of Sweden (SGU), 44% of the around 19,000 private wells that have been sampled in their surveys had manganese concentrations below 50 µg/L. Around 85% had manganese concentrations below the recommended guideline value of 300 µg/L and 90% had manganese concentrations below the WHO guideline value of 400 µg/L. Concurrently, 10% of the sampled wells had manganese concentrations above

400 µg/L and in 2% of the wells, manganese concentrations were above 1 mg/L. Figure 2 shows the distribution of manganese concentrations in all of the sampled wells, as well as the distribution according to well type. The data originate from eight different surveys carried out by SGU, which should minimize bias with regard to sample selection.

The SGU data also showed large variations in manganese concentrations depending on well type. A small number, 4%, of the sampled wells retrieve water from springs, most of which had manganese concentrations below detection limits. Almost one fourth of the wells are dug while 70% are drilled. The median manganese concentrations in these wells were found at 20 and 80 µg/L, respectively.

2 Human exposure

Manganese is an essential element and is needed for catalytic activity or activation of several enzymes (Korc, 1988). Manganese is required for normal amino acid, lipid, protein and carbohydrate metabolism and it is needed by the fetus to support normal growth and development (Dorman et al., 2006). It is crucial for maintaining the proper function and regulation of many biological processes such as producing ATP and blood clotting. It is utilized by various antioxidant enzymes such as superoxide dismutase (MnSOD) and activates the glycosyltransferase necessary for the mucopolysaccharides utilized by cartilage, bone and other connective tissues (Erikson et al., 2007).

According to Levy & Nassetta, (2003) inhalation is the most important route of entry in most occupational settings. In the non-occupational environment, ingestion of manganese through food is the major exposure route, with daily intake ranges for adults estimated at 0.9-10 mg manganese (Klaassen, 1996; ATSDR, 2000). According to Pennington & Young (1991), grains, beverages (especially tea) and vegetables provide approximately 33%, 20% and 18%, respectively of dietary manganese in adult males. Rice and nuts also contain significant amounts of manganese. According to the Swedish National Food Administration (Livsmedelsverket, 1996), wheat sprout and wheat bran contain the highest concentration of manganese of the investigated food stuffs, about 180 and 120 mg manganese per kg edible product, respectively. Figure 3 shows

the manganese concentrations of different food stuffs in Sweden. Cereals, breads and nuts contain the most manganese, while very little is found in meat, fish and seafood. Berries and vegetables also hold significant amounts. Although coffee shows the higher value (7.7 mg/kg) among the investigated drink types in figure 3, it should be noted that it refers to instant coffee powder, and not to the prepared drink. When prepared with water, a cup of tea contains somewhat more manganese (about 1.6 mg/kg) compared to an instant cup of coffee (about 1.1 mg/kg).

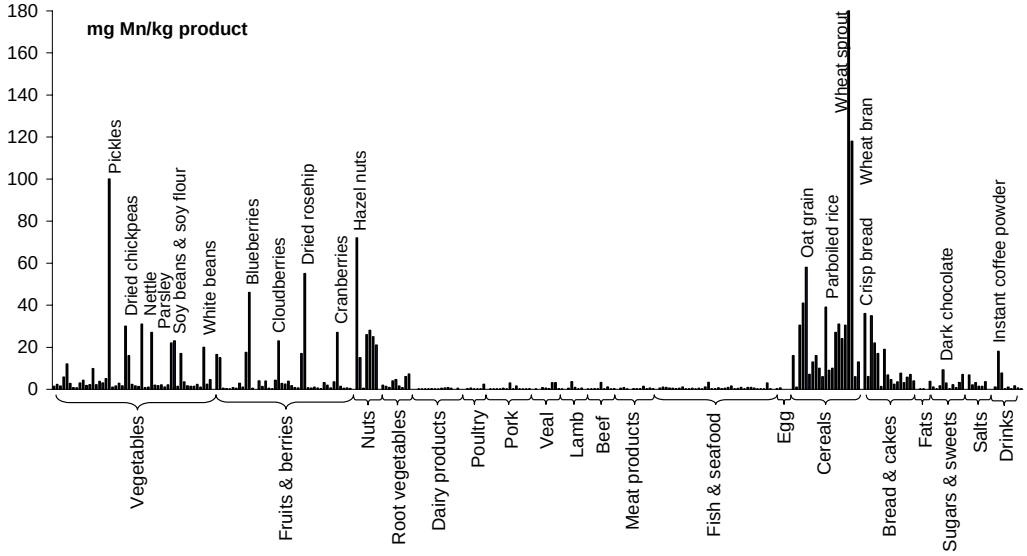

Figure 3. Manganese concentration (mg Mn/kg edible part) in different Swedish food stuffs (Livsmedelsverket, 1996).

Existing knowledge on manganese metabolism and consequences of too low intakes were considered insufficient by the authors of the Nordic Nutrition Recommendations of 2004 (NNR, 2004) for setting requirements and recommended daily intakes. They refer to a report by the Food and Nutrition Board (IOM, 2002) where it is suggested that a daily intake of 740 µg manganese should be enough to replace daily losses. The IOM (2002) were also not able to set a recommended daily intake (RDI) for manganese but has instead set an adequate daily intake level (ADI) at 1.8 mg for adult women and at 2.3 mg for adult men. The EU Scientific Committee on Food also acknowledged the lack of information on manganese toxicity and suggested

that the current population intake in the EU is adequate. A safe and adequate range of daily intake was set at 1-10 mg (SCF, 1993).

Ingestion of water usually constitutes a minor exposure route. However, when drinking water contains elevated levels of manganese, this source of exposure may be of significance. It has been recognized specifically as an important source of exposure to infants receiving infant formula (Sievers, 2005).

Regardless of broad day-to-day variations in oral manganese intake, adult humans generally maintain a stable manganese tissue level because the gastrointestinal absorption as well as the hepatobiliary excretion are strictly regulated (Aschner et al., 2005; Dorman et al., 2006). In infants, however, this homeostasis is not yet fully developed, and manganese intake is therefore not regulated. The following section will focus on children's exposure to manganese, since it differs from that of adults, both with regard to sources of exposure and susceptibility.

2.1 Children's exposure

Children's exposure to manganese differs from that of adults. Both their behaviour and their physiology influence the extent of exposure and any potential adverse health effects. Their exposure and susceptibility also depends on development stage and age, nutrition status and exposure to other elements and pollutants (ATSDR, 2000).

While the main source of manganese in the general adult population is food, infants and young children often do not consume food containing elevated manganese concentrations, such as tea, leafy vegetables, fruits, cereals and nuts. Instead, their main nutrient intake, including manganese, occurs via their mother's milk or a substitute for this, such as infant formula. Infant formulas are often distributed in powder form and must be mixed with water prior to ingestion. Manganese in drinking water of their homes may therefore also become an important source of manganese for infants and young children. Complementary foods are usually introduced to the infant at about four months of age which may add significant sources of manganese. Moreover, as the child becomes more mobile with age, their hand-to-mouth behaviour may introduce additional sources of manganese.

Premature children are often given parenteral nutrition which holds significant concentrations of manganese making it an important manganese source for this group of children. Manganese exposure also occurs prior to birth when fetuses are exposed through transport across the placenta.

2.1.1 Prenatal exposure

Both in vitro human and in vivo rodent studies have shown that the transport of manganese across the placenta is low and that the human placenta accumulates manganese (Osman et al., 2000; Aschner et al., 2005). In spite of this, toxic effects have occurred in fetus of rodents in the absence of maternal toxicity after oral exposure to high manganese levels. The mechanism involved in manganese distribution across the placenta is currently unknown (Dorman et al., 2006). Aschner et al. (2005) suggest that both transferrin and the divalent metal transporter 1 (DMT-1) may be involved. They are both present in the placenta and are upregulated in maternal iron deficiency, so that the fetus is seldom severely affected by anemia. Considering the potential shared function of transferrin and DMT-1 in iron and manganese transport, maternal Fe deficiency may lead to increased manganese placental transport to the fetus.

Krachler et al. (1999a) found that the manganese concentration of umbilical cord sera was 150% higher than that in the corresponding maternal sera. The study also investigated colostrum manganese concentrations which were found at around twice the concentrations in both umbilical cord and maternal serum. Rossipal et al. (2000) also found higher manganese concentrations in the umbilical cord sera of newborns (150%) as well as in the colostrum (275%) compared to maternal sera. Because of the significantly higher manganese concentration in the sera of the umbilical cord compared to the maternal concentration, Krachler and co-workers suggest that there likely is an active transfer of manganese in the mammary gland and in the placenta. They were not, however, able to establish a correlation between manganese concentrations in newborns and their mothers.

2.1.2 Parenteral exposure

Premature infants and children that suffer from e.g. liver disease often receive parenteral nutrition. Most parenteral nutrition solutions contain manganese since manganese is needed for normal brain development and functioning. However, manganese toxicity can occur (Stobbaerts et al., 1992). According to Fell et al. (1996) and Aschner et al. (2005), children receiving parenteral nutrition are at risk of both liver and neurotoxicity. Fell and co-workers reported abnormalities of the basal ganglia in four children on long-term parenteral nutrition (>2 years) as well as associations between high blood manganese concentrations and hepatic disease. During cranial MRI, Quaghebeur et al. (1996) also detected abnormalities in 7 children on long-term parenteral nutrition. Fell et al. (1996) concluded that manganese toxicity was an important factor for cholestatic liver disease, which complicates parenteral nutrition, especially in children although there are other important factors as well.

The manganese concentrations of parenteral nutrition solutions ranged from 5.6 to 8.9 µg Mn/L in a study conducted in 1992 (Wilson et al., 1992). Stobbaerts et al. (1992) calculated the daily manganese intake from total parenteral nutrition from 10 randomly chosen solutions and found a mean value of 5 mg/day. Peditrace® is a supplement used in Sweden, intended to cover basal needs of trace elements in new-born infants and children on parenteral nutrition. The daily dose for infants <15 kg is set at 1 ml/kg body weight and the manganese concentration is 0.001 µg/L (Fass, 2003). Another used supplement is Tracel®, with a concentration of 0.027 µg Mn/L and a recommended daily dosage of 0.1 ml/kg (Fass, 2005). These supplements would thereby provide around 3-8 µg manganese daily for a 3 kg infant.

In children on total parenteral nutrition, the homeostatic barrier that normally regulates manganese absorption is bypassed. At the same time, these children often have hepatic dysfunction and cholestasis which comprises their ability to excrete manganese via the bile. These factors may allow for more manganese to enter the developing brain than would occur in adults (Erikson et al., 2007).

2.1.3 Human milk

According to ATSDR (2000), the manganese concentration in human milk ranges from 3.4 to 10 µg/L. The European Commission's Scientific Committee on Food (SCF, 2003) based their calculations of infant manganese intake on a study by Casey et al. (1985) who found an average manganese concentration of 3.5 µg Mn/L in human milk. The SCF used this value in their revision of infant formula requirements and calculated the manganese intake of breast-fed infants at 2.5-3 µg/day.

The manganese concentration in human milk seems to be related to the stage of lactation, as the concentration decreases with time. Stastny et al. (1984) found mean manganese concentrations of 6.6±4.7 µg/L in lactation week 4, which were significantly higher than in the 12th week (3.5±1.4 µg/L). The study included 24 mothers. Vuori et al. (1980) also found decreasing manganese milk concentrations of 15 mothers studied during two one-week periods. The first period ranged from week 6 to 8 after birth when the milk manganese was 4.5±1.8 µg/L. In weeks 17 to 22, the mean manganese concentration was slightly lower at 4.0±1.5 µg/L. In the study by Casey et al. (1985) manganese concentrations were 5.4±1.6 µg/L on day one and decreased to 2.7±1.6 µg/L on day 5. Between day 8 and 28 the manganese concentration was rather constant at a mean of 3.7±2.2 µg/L. The authors calculated an average daily intake value of 2 µg by the infants over the first month of life using 24-h test-weighing measurements.

A study by Vuori and coworkers (1980) found a significant positive correlation between manganese intake via food and breast milk concentration in 15 breast-feeding mothers. The correlation was observed during the second survey week, which ranged between 17-22 weeks after delivery, but not between weeks 6-8. Al-Awadi and Srikumar (2000) analyzed manganese in breast milk of Kuwaiti and non-Kuwaiti women living in Kuwait at three different stages during lactation (0-6 months, 6-12 months, 12-18 months). They observed a decrease in manganese concentration with time in both groups of women. The Kuwaiti women had slightly but not significantly higher manganese concentrations (6.0, 4.2, 3.8 µg/L in months 0-6, 6-12 and 12-18, respectively) than the non-Kuwaiti women in the study (5.7, 3.7, 3.1 µg/L in months 0-6, 6-12 and 12-18, respectively). The authors also compiled information on manganese concentrations in breast milk of mothers in different countries. Studies

performed in Austria, Germany, Italy and Korea showed similar manganese milk concentrations as previous studies reported above (3.1, 6.2, 4.1, 2.7-4.0 µg/L, respectively). No studies have been found on the milk manganese concentrations of Swedish women.

Table 1. Breastfeeding frequency (%) in Swedish children born 2004 (Socialstyrelsen, 2006)

Age	Exclusively breastfed [a]	Partially breastfed [b]	Not breastfed
1 week	89.4	8.6	2.1
2 months	77.3	14.1	8.6
4 months	63.8	18.9	17.3
6 months	19.2	52.8	28.0
9 months	1.0	40.5	58.5
12 months		19.6*	80.4

[a] Additional daily intake from vitamins and medicines only
[b] Additional daily intake from formula and taste-portions of food
* Both partially and exclusively breastfed children

Nearly all (98%) of the Swedish children born 2004 were exclusively breastfed during their first week of life. At the age of two months, 91% were exclusively or partially breastfed, while only 9 % were not breastfed at all. Swedish recommendations state that taste portions should be introduced at the age of four months, and a marked decrease in number of children exclusively receiving breast milk is noted between the ages of four and six months. Table 1 shows data on breastfeeding practices in Sweden in children born 2004.

2.1.4 Infant formulas and follow-on formula

Although most children are exclusively breastfed at an early age in Sweden, there is a fraction of children that may be allergic to substances in human milk, whose mothers cannot breastfeed them, or to whom breast milk is not available. In order for these children to attain adequate nutrient intakes, infant formulas have been developed. As the child grows, it may also need complementary nutrients from follow-on formulas. The EU Infant Formulae Directive (SCF, 2003) defines infant formula as foodstuffs intended for use by infants during their first four to six months of life, while foodstuffs intended for infants aged above four months and young children is termed follow-on

formula. While infant formula is intended to provide the sufficient nutrition requirements on its own, follow-on formula is intended to constitute the principal liquid element of a child's diet as it becomes progressively more diversified (SCF, 2003).

Table 2. Manganese concentration in powdered infant formula and follow-on formulas (µg/L) (R = reflux, G = gluten intolerance, C = cow milk protein intolerance, S = soy protein allergy, L = lactose intolerance, P = parenteral nutrition, M = malabsorption).

Product	Intended subject	Base	Mn (µg/L)	Mn (µg/100 kcal)
		From birth		
NAN 1[1]	All	Milk	50	7.5
Baby Semp[2]	All	Milk	25	3.8
Enfamil AR, Lipil[3]	R G	Milk	410	60
Neocate[4]	C M S	Free amino acids	600	85
Profylac[2]	C	Hydrolyzed whey	400	64
Pepti-Junior[4]	C S M	Hydrolyzed whey	400	60
Pregestimil[3]	C S L M	Hydrolyzed casein	410	60
Nutramigen[3]	C S L	Hydrolyzed casein	410	60
Nutramigen 1, LGG[3]	C S L	Hydrolyzed casein	410	60
Prosobee[3]	C L	Soy	410	60
MiniMax Soja[5]	C L	Soy	300	45
		From 4 months		
NAN 2[1]	All	Milk	50	7.5
BabyPlus[2]	All	Milk	25	3.6
Nutramigen 2 med LGG[3]	C S	Hydrolyzed casein	430	60
		From 6 months		
Bifidus[2]	G	Milk	25	3.8
Lemolac[2]	*acidified*	Milk	25	3.8
MiniMax Barnsondnäring[5]	P	Milk	700	58

[1] Nestlé [2] Semper [3] MeadJohnson Nutritionals [4] Nutricia [5] Novartis

According to Lönnerdal (1994), infant formulas based on cow's milk hold 30-50 µg/L while soy-based formula contain 200-300 µg/L. For comparison, human milk contains on average 4-6 µg Mn/L. Table 2 shows the most common brands of infant formula and follow-on formulas found in Sweden. There are more infant formulas on the market, but it was not possible to find the manganese concentrations of those products. The formulas based on cow's milk which are intended for children without any particular intolerance hold either 25 or 50 µg Mn/L prepared product, assuming that the water used for preparation does not any contain manganese. The remaining formulas are intended for infants with various intolerance disorders and allergies and are based on either soy or milk. The milk-based formulas are comprised of either hydrolyzed whey or casein, instead of milk proteins. This is because the size of the protein determines their ability to induce an allergic reaction. Through the hydrolyzation process, large milk proteins are split into smaller molecules and separate amino acids which will not cause an allergic reaction (Kjellman & Oldaeus, 1998). Most soy and milk based allergy formulas hold 410 µg Mn/L, but the concentration ranges between 300 and 600 µg Mn/L.

Soy beans contain higher manganese concentrations than milk by nature, which explains the 10-fold higher manganese concentration in the soy formula compared to the milk based formula. However, the milk based formulas intended for allergic or intolerant infants contain similar manganese concentrations as the soy based products.

2.1.5 Drinking water

Children may be exposed to manganese in drinking water through both direct consumption, and through its use for preparing powder-based infant formula and follow-on formula or any other foods prepared from water, as has been described above. While the domestic tap water quality may be of negligible importance for the manganese exposure of exclusively breastfed infants, it is essential for infants who receive infant formula as well as for young children who receive complementary foods (Sievers, 2005). An infant's intake of water in relation to body weight is considerably higher than for adults. WHO estimates a daily water intake of 150 ml/kg for infants and 33 ml/kg for adults (Sievers, 2005). The German reference values for water intake is set at 120 ml/kg for infants and at 35 ml/kg for adults (DACH, 2000 *cit* Hilbig et al.,

2002). While adults have a total body water concentration of 50-60%, the body of an infant contains 70% water. The daily turnover rate is also higher in infants, around 20% compared to around 6% in adults. According to a study described in Sievers (2005), full-term exclusively formula-fed infants consume 0.5-1.2 L drinking water per day at the age of 2 months, 0.5-0.8 L/day at 4 months and 0.3-1 L/day at the age of one year. For preterm infants, the intake was slightly higher at 2 and 4 months and slightly lower at one year of age.

Because of the relatively large water intake in infants, manganese present in water may contribute significantly to the infant's total daily manganese intake (Sievers, 2005). The European Commission's Committee on Food Safety has recognized the importance of water quality when preparing powdered formula (SCF, 2003). However, their recommendations of mineral concentrations in infant formula refer to the total concentration as prepared for consumption, and do not regard the water quality itself (Sievers, 2005). Likewise, the manganese concentrations provided by the producers on the labels of infant and follow-on formulas do not consider additional manganese from water used for preparation.

3 Kinetics and metabolism

Ingested manganese is subjected to delicate homeostatic control, since it is an essential element that the body needs for its well-being. After absorption in the gastrointestinal tract, manganese is rapidly cleared from the blood by the liver and excreted in bile. Its concentration must be contained within certain limits for optimal functioning; both deficiency and toxicity symptoms have been observed (Mergler, 1999). Symptoms of manganese deficiency have been observed in experimental animals, but are generally not recognized in humans because of the widespread presence of manganese in the human diet (Aschner et al., 2005).

Several studies have shown that the manganese concentration in the cord blood of newborns is significantly higher than that of adults (Krachler et al., 1999a; 1999b; Rükgauer et al., 1997). It has been suggested that the manganese absorption in infants is greater than that in adults because of the not fully developed homeostasis in infants (Keen et al., 1986). Moreover, manganese

retention has been suggested to be higher in infants because of the low bile flow, limiting excretion (Lönnerdal, 1994). Because of the not fully developed nervous system of infants, they are also more susceptible to manganese toxicity. The following sections will therefore have a special focus on manganese intake with regard to infants and young children.

3.1 Absorption

Only a small fraction, between 1 and 5%, of ingested manganese is normally absorbed from the gastrointestinal tract. The uptake is regulated so that when dietary manganese levels are high, the gastrointestinal absorption is reduced (Klaassen, 1996; Aschner et al., 2005). The absorption mechanism from the gastrointestinal tract is not completely understood (Aschner et al., 2005) and while some studies suggest that manganese absorption occurs via passive diffusion transport (Bell et al., 1989), others suggest an active transport (Garcia-Aranda et al., 1983). Manganese absorption is influenced by the iron status as well as the status of other nutrients and minerals. While manganese absorption increases with iron deficiency (Finley 1999), it decreases with calcium supplementation (Freeland-Graves & Lin, 1991). According to Aschner et al. (2005), the resulting increase in manganese absorption from Fe deficiency may lead to an enhanced delivery of manganese to the brain. At the same time, manganese absorption decreases with an increased Fe intake. Manganese absorption is also affected by the carbohydrate source in the diet, the presence of phytate and of animal protein (Finley, 1999).

Absorption of manganese is affected by the presence of other elements in the diet. By using extrinsic labeling of test meals with a manganese radioisotope and whole-body retention measurements, Davidsson et al. (1991) investigated how adult manganese absorption from human milk was affected by added calcium and manganese. They also investigated how manganese absorption from infant formula is affected by added calcium, phytate, phosphate and ascorbic acid, and how absorption from wheat bread is affected by added iron and magnesium. The only test meal that affected manganese absorption was the added calcium to human milk which resulted in a significant decrease in absorption. Finley (1999) found in a study of young women that their iron status as measured by ferritin concentration, affected manganese absorption: the greatest absorption (5%) was observed in women with low ferritin

concentrations who consumed low manganese diets. These women absorbed almost five-fold more manganese than women with high ferritin concentrations consuming the same diet. The study was carried out on adults. In an earlier study, Finley et al. (1994) showed that there was a significant association between manganese absorption and serum ferritin concentrations in women but not in men. They also found that women had lower total serum ferritin concentrations than men.

3.1.1 Infant absorption

No exact data on manganese absorption in newborn humans exist but studies of gastrointestinal absorption of other metals, such as Pb have shown that it is greater in infants and in young children than in older ones. The difference in pharmacokinetics may be due to the immature gastrointestinal tract of newborns and their larger gastrointestinal skin surface area in proportion to body weight, facilitating a relatively a higher absorption (ATSDR, 2000). According to Lönnerdal (1997), the number of lactoferrin receptors per tissue weight is highest during infancy, although it is present at all ages, including the fetus.

Since there is no stable isotope available for manganese, and the use of radioisotopes in children is not convenient, it is very difficult to determine manganese absorption in infants accurately. Because of the very low levels of manganese in both breast milk and infants' urine and faeces, it is also difficult to make accurate measurements. Moreover, there is no possibility to quantify endogenous losses of manganese, further complicating determinations of true absorption (Lönnerdal, 1994). Because of the many difficulties with determining the manganese absorption in infants, most studies have been carried out on experimental animals.

Dorman et al. (2005) exposed neonatal rats and their dams to different concentrations of $MnSO_4$ through inhalation. The study found that the blood manganese concentrations in the new-born pups were very high but returned to similar concentrations as found in the control group by postnatal day 19. This initial spike in blood manganese concentrations was surprisingly found to be due to manganese intake through milk rather than $MnSO_4$ inhalation. When stomach concentrations of one day old pups were analyzed for manganese, the

milk manganese concentrations were found at 2-8 fold higher than in air-exposed control pups. Keen et al. (1986) also showed that infant rats absorbed significantly more manganese from the gut than mature animals. They administered human milk labeled with ^{54}Mn to rat pups younger than 15 days of age. Around 80% of ingested manganese was retained after 6 hours. In older pups, 40% of the oral dose was retained.

Dörner et al (1989) determined infant absorption by conventional balance technique and found that around 20% of the manganese in formula fed infants was absorbed. This value is considerably higher than those found for human adults where manganese retention values, determined by radioisotope methods and whole body counting, have been determined at 2-8% for most diets (Lönnerdal, 1994).

3.2 Distribution

At physiologically appropriate manganese intake levels, manganese specifically concentrates in mitochondria. Tissues rich in mitochondria, such as bone, liver and pancreas therefore tend to have higher manganese concentrations than other tissues. The largest tissue store of manganese is in the bone structure and the biological half-life of manganese in the body is 37 days (Klaassen, 1996). With chronic intake of excess manganese, the manganese concentration in mitochondria increases, although the relative fraction with regard to distribution is not changed (Aschner et al., 2005). Dobson et al. (2004) have reviewed manganese toxicity studies of humans and macaque monkeys using MRI, and found that manganese concentrations were highest in parts of the brain that are usually rich in iron. According to the authors, it appears as if iron deficiency may lead to manganese accumulation in these brain regions (Dobson et al., 2004). Liver especially accumulates manganese after high exposures, which is why liver disease is a risk factor for manganese accumulation in the brain (Dobson et al., 2004).

In plasma, 80 % of manganese is bound to small molecular weight carriers such as β-globulin, albumin and citrate, and a small fraction is bound to transferrin, the primary binding and transport protein for Fe (Aschner et al., 2005). Manganese is exclusively in its trivalent state when complexed to transferrin (Erikson et al., 2007). As a divalent cation, manganese is able to

bind to negatively charged ions such as nitrate, phosphate and carboxylate groups. It is thereby able to react with proteins and can inhibit and activate various enzymes. Manganese is also a necessary co-factor for some metalloenzymes or manganoproteins such as the manganese superoxide dismutase (MnSOD) and the glutamine synthetase (GS) (Vernole et al., 2003). MnSOD is one of the most important antioxidant enzymes while GS plays an essential role in the metabolism of nitrogen. The DMT-1 is responsible for the uptake of Fe^{2+} from dietary sources in the intestinal lumen, and is independent of transferrin. It also acts as an exporter of Fe^{2+} which has been released from transferrin in acidified endosomes. (Crossgrove & Yokel, 2004). According to Conrad et al., (2000), manganese and iron compete for uptake via DMT-1 in human leukemia and kidney cell lines. At present, the function of DMT-1 in manganese uptake is not clear. Duodenal absorption of metals seems to be DMT-1 regulated, and although it is present in newborns, it does not seem to be Fe-regulated until adulthood (Erikson et al., 2007).

3.2.1 Distribution into the brain

Manganese can be transported across the blood brain barrier via both active and passive mechanisms where its half-time is longer than in the whole body. Manganese is eliminated from the brain over time with a half life of 50-75 days in rodents and in non-human primates (Dorman et al., 2006).

Transport of manganese into the brain has been suggested to occur via several different mechanisms, including facilitated diffusion, active transport, DMT-1 mediated transport, ZIP8 transport, store-operated calcium channels as well as tranferrin-dependent transport (Erikson et al., 2007). Since transferrin is present on the surface of capillaries in the brain (cerebral cortex), it has been suggested that manganese enters endothelial cells in the trivalent form complexed with transferrin. The trivalent manganese is then released inside the cell and transferred to the cell surface where it is released to the extracellular fluid, transported by brain derived transferrin and taken up by neurons that possess both transferrin receptors and DMT-1. The findings that manganese is accumulated in the same areas of the brain as iron, support the theory of transferrin being an important transporter for manganese (Erikson et al., 2007). DMT-1 has also been suggested as an important transporter of manganese into the brain. However, a recent study of manganese transport across the blood-

brain barrier, found no differences between Belgrade rats and control rats. Since the Belgrade rat lacks significant amounts of functional DMT-1 it was concluded that DMT-1 is not a major transporting mechanism for manganese into the brain. (Crossgrove & Yokel, 2004). Crossgrove et al. (2003) found that manganese transport into the brain was carrier-mediated and that manganese citrate entered the brain more rapidly than manganese bound to transferrin and the divalent manganese ion. Crossgrove & Yokel (2005) suggest that the mediated carriers are store-operated calcium-channels.

According to Erikson et al. (2007) several in vitro studies have shown that trivalent manganese does not accumulate intracellularly to such significant amounts as to lead to neurotoxicity. It has instead been suggested that it is the inhibition of Ca^{2+} activation and control of ATP caused by Mn^{2+} that causes neurotoxicity. Mn^{2+} binds to Ca^{2+} binding sites stronger than Ca^{2+} itself, inhibiting the Ca^{2+} activation of ATP production. Since cells in the basal ganglia require ATP for maintaining its proper functions, this results in lowered cell function and hence neurotoxic effects (Gunter et al., 2006).

3.2.2 Distribution in children

Keen et al. (1986; 1999) and Takeda et al. (1999) suggest that high amounts of manganese are required for normal brain development in infants. However, excessive manganese intake may have neurotoxic effects. In children and neonatal rodents, manganese accumulates preferentially in certain regions of the brain (Zlotkin & Buchanan, 1986; Kontur & Fechter, 1988). Several studies have shown that neonatal rodents develop higher brain manganese levels than adults following similar oral exposures (Dorman et al., 2006). Results from a study by Dorman et al. (2000) suggested that manganese was more efficiently transported into the CNS of neonates compared to adults receiving equal oral doses of manganese. They compared neonatal and adult rats that had been exposed orally to identical $MnCl_2$ doses for 21 consecutive days. Increased brain manganese concentrations were observed in all exposed neonates on postnatal day 21 while adult rats had fewer changes. This tendency of neonates to achieve higher brain manganese concentrations may reflect their less than complete blood-brain barrier and/or markedly reduced biliary manganese excretion rates (Aschner et al., 2005).

3.3 Excretion and retention

Although a small proportion of manganese is excreted in sweat, hair, milk and urine, biliary excretion is the main route of manganese removal from the body. Manganese is removed from the blood in the liver, conjugated with bile and excreted into the intestine, where a small fraction is re-absorbed. A stable body manganese level is maintained by a tightly controlled regulation of absorption and excretion via bile (Aschner & Aschner, 2005).

3.3.1 Excretion and retention in infants

The retention of manganese in the human body, including both absorption and excretion, differs with age. Several studies have found that newborns and infants have higher manganese levels than older children or adults. According to Lönnerdal (1994) the bile flow is low in infants, which may result in a higher retention and deposits at various sites in the body. Moreover, certain tissue sites have a high affinity for manganese, and while these sites are saturated in adults resulting in excretion, they strongly retain manganese in infants. A reason for this may be the requirement of manganese for adequate connective tissue synthesis.

A Japanese study on erythrocyte manganese levels in 165 healthy children, 40 adults and 10 elderly persons found that the mean manganese concentrations in infants at the age of one month (121±25 µg/L) were three to four times higher than that found in adults. After one month of age, the manganese concentration decreased rapidly and was constant at 44±8 µg/L between the age of four months and 11 years. At 12 years, the manganese concentration of males decreased further to significantly lower concentrations than found in females (33±5 and 44±8 µg/L, respectively) (Hatano et al., 1983). The difference between males and females is likely due to Fe deficiency of women at fertile age. The results from this study are supported by Rükgauer et al. (1997) and Krachler et al. (1999b) who found a decrease in serum manganese concentrations with age when comparing 137 and 117 individuals of different ages, respectively. Alarcon et al. (1996) also investigated serum manganese concentrations in children of different ages, in total 180 infants were examined. The children ranged from 5 days to 12 months of age and were all residents of the same town in Venezuela. Alarcon and co-workers also found a decrease in

blood manganese concentrations with age, although the decrease was not as marked as for the Japanese children.

Studies measuring head hair manganese concentrations in 418 children and adolescents aged 6 months to 20 years also found decreasing concentrations with increasing age (Sakai et al., 2000). Although hair manganese is not a very good biomarker of manganese status because of the many external factors that affect hair manganese concentrations, their results further support the findings described above of higher manganese concentrations in young infants and a decrease with age. This difference is likely due to both a higher absorption and a lower excretion of manganese, although the exact mechanisms for these processes are not known.

Another possible explanation for the findings of decreasing blood manganese concentrations with age could be a higher manganese concentration of infants at birth. Samples of cord blood have shown significantly higher manganese blood concentrations in cord blood than in maternal blood. Both Rossipal et al. (2000) and Krachler et al. (1999a) found that the manganese concentration of umbilical cord sera was 150% higher than the corresponding maternal sera. Takser et al. (2003; 2004) have performed two studies of cord blood manganese concentrations. In 2003, cord blood was collected from 222 newborns with a geometric mean manganese concentration of 39 µg/L, ranging from 15-93 µg/L. In 2004, cord blood was collected at 87 deliveries. The manganese concentrations were 34 ± 13 µg/L with a range of 17-90 µg/L (Takser et al., 2004). These values can be compared to a reference range of 5.0-12.8 µg/L in blood of 100 healthy volunteers assessed by Goullé et al. (2005) who also found a median value of blood manganese at 7.6 µg/L.

3.4 Manganese bioavailability from infant foods

The bioavailability of manganese in infant foods will determine its absorption and retention in the infant body, and hence any effects of toxicity or deficiency. Infants mainly consume either human milk or formula, why the forms of manganese present in human milk, cow's milk, soy milk and water are relevant for their manganese exposure. Several studies suggest that manganese in formulas is less bioavailable than manganese in breast milk (Stastny et al., 1984; Davidsson et al., 1989; Lönnerdal, 1989). Stastny et al. (1984) found that

in spite of consuming a larger volume of milk, formula-fed infants had similar mean sera manganese concentrations as breast fed infants (4.7 and 4.4 µg/L, respectively). The formula fed infants consumed ~900 ml/day, compared to ~700 ml/day for breast fed infants, which in combination with the higher manganese concentration of formulas resulted in a significantly higher manganese intake – 180 µg/kg/day compared to 0.4 µg/kg/day for breast fed infants. The similarity in sera manganese concentrations in spite of the difference in intake indicates a much lower bioavailability of manganese in formula compared to that in human breast milk.

3.4.1 Manganese distribution

The distribution of manganese in milks and formulas differ significantly. Lönnerdal et al. (1985) found that while manganese was predominantly found in the whey fraction of human milk (71%), it was found mainly in the casein fraction of cow milk (67%) and soy formula (54%), and in the fat fraction of the cow milk formula (65%). Al-Awadi & Srikumar (2001) found similar results with manganese mainly bound to whey (66%) in human milk and mainly to casein (62%) in cow milk. Figure 4 shows the manganese distribution between fat, casein and whey in the four different milk types, as well as the distribution between different whey proteins. The figure also shows the distribution of humanized cow's milk formula, where bovine whey proteins have been added in order to more closely resemble the distribution of human milk (Lönnerdal et al., 1985).

Lönnerdal et al. (1985) found that over half of the manganese in human milk was bound to lactoferrin, compared to only 10% in cow's milk. Brätter et al. (1998) also found that manganese in human milk was mainly associated with lactoferrin (65-70%) with the remaining part bound to low molecular weight compounds (LMW). In the infant formulas analyzed in their study, manganese was mainly bound to LMW. The binding of manganese to lactoferrin in human milk facilitates absorption since an intestinal receptor exists for lactoferrin, enabling a higher uptake than from formulas where manganese is bound to LMW (Lönnerdal, 1989). In contrast to the two studies presented above, Michalke & Schramel (2004) found that almost the total manganese fraction in human milk whey was present in the LMW fraction. Martino et al. (2002) also

found that in both human and formula milk whey, manganese was predominantly (86-95%) in the LMW fraction.

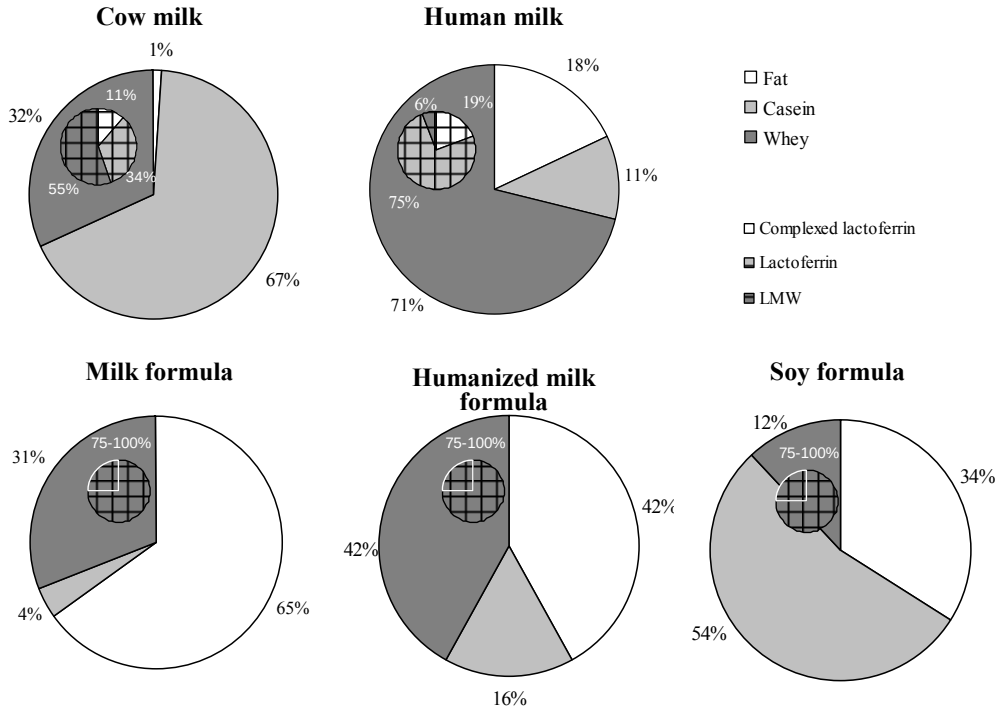

Figure 4. Manganese distribution in cow's milk, human milk, cow's milk formula, humanized cow's milk formula and soy formula (after Lönnerdal et al., 1985).

One reason for this difference in distribution may be the time of sampling as human milk composition changes with lactation time. According to Lönnerdal (1997) the whey-to-casein ratio in human milk at early lactation is 80:20, which is the opposite from cow's milk. Because of this natural difference in whey-to-casein ratio, some formula manufacturers have added bovine whey protein to cow's milk in order to more closely resemble human milk. As human lactation continues, the ratio is changed from 70:30 to 60:40, and may reach a 50:50 ratio in the milk of women who breastfeed for a relatively long time. In the Michalke & Schramel study, milk was collected on the third day after delivery, while the Lönnerdal study used milk from mid-lactation at 2-4

months after delivery. The Al-Awadi & Srikumar study used milk from a lactation period of 2 weeks to 18 months.

In a study by Davidsson et al. (1989), the fractional absorption of manganese in adults from different infant foods was investigated. The authors found that while the absorption from human milk and cow's milk were 8.2 and 2.4%, respectively, the absorption from soy formula was significantly lower, at 0.7%. A factor which may limit the bioavailability of manganese from soy formula is the high proportion bound to casein. Soy formulas also contain phytate which may limit manganese uptake (Lönnerdal, 1989). Since iron and manganese share common uptake pathways, ratio imbalances in formulas may further limit bioavailability (Lönnerdal, 1989).

3.4.2 Manganese speciation

In anaerobic conditions in the environment, such as those found in deep wells, manganese is reduced to its more soluble divalent form. The manganese found in the water that is mixed with infant formula is therefore often in its divalent state (BGS & WaterAid, 2003). The manganese found in infant formula is also found in its divalent state while manganese in breast milk is present in its trivalent form. The transporter transferrin binds to manganese exclusively in its trivalent form (Aschner et al., 2005), thereby regulating manganese uptake. In formula and water where manganese is present in its divalent form, this regulation of absorption by transferrin receptors is absent and other mechanisms are likely to govern the transport of manganese (Erikson et al., 2007).

4 Biomarkers

Biomarkers are used as indicators of exposure and may be useful in assessing risks of negative effects from excessive intake. While biomarkers of effect are used to measure biochemical, physiological or any other alterations to establish negative health effects, biomarkers of exposure are used to assess the dose that an individual has been exposed to. The latter types will be discussed in the following sections.

4.1 Urine

Urine is generally a bad indicator of manganese exposure. It represents only 1% of the daily absorbed amount and 6% of the total excreted amount (Smargiassi & Mutti, 1999). However, while studies on both female and male volunteers have failed to find correlations between manganese exposure and manganese concentrations in urine, significantly increased levels of urinary manganese have been reported in occupationally exposed male workers (Aschner et al., 2005).

4.2 Blood

According to Lönnerdal (1994), whole blood provides a reasonable estimate of long-term manganese status while plasma and serum analyses are more reflective of short-term intake than of long term status. Blood manganese changes very little with exposure, why it doesn't indicate recently absorbed amounts (Smargiassi & Mutti, 1999). According to Greger (1999) several researchers have found an accumulation of manganese in blood from patients provided with supplemental manganese in parenteral nutrition and in patients with impaired liver function. Hatano et al. (1985) found differences in manganese erythrocyte levels between formula-fed and breastfed infants, where the formula fed infants who ingested more manganese, had higher manganese levels. Stastny et al. (1984) however, found no significant differences in serum manganese concentrations of breast fed and formula fed infants, in spite of large differences in manganese intake. Similarly, no differences in serum manganese concentrations were found by Kondakis et al (1989), who studied persons exposed to different concentrations of manganese through their drinking water. Erythrocyte manganese may be a better biomarker of manganese in blood than serum manganese, since most of the manganese in blood is associated with the red blood cell. According to Lönnerdal (1994), plasma manganese levels are also often very low (1-5 μg/L) compared to whole blood levels (10-15 μg/L) which may add difficulties to accurate manganese measurement.

Another reason for the limited value of plasma or serum when assessing an individual's long term manganese status is the strong association between iron and manganese. The absorption of manganese may be increased in iron

deficient persons, and as manganese is excreted in the bile, the serum manganese level may not reflect the manganese status (Lönnerdal, 1994).

4.3 Hair

Hair samples can be used to reflect manganese exposure over a matter of months (Erikson et al., 2006). Woolf et al. (2002) found elevated blood and hair manganese concentrations in a boy chronically exposed to high manganese concentrations from drinking water. His brother, who showed no symptoms of manganese toxicity, also had elevated hair manganese levels, but his blood manganese levels were within the normal range. Bouchard et al. (2007) used hair as a biomarker for manganese exposure in school-aged children and found significant correlations between manganese concentrations in their hair and in the tap water at their homes. Collipp et al. (1983) also found significantly higher hair manganese in infants who consumed more manganese through their diets. Kondakis et al. (1989) also found differences in hair manganese between three groups being exposed to different manganese concentrations through their drinking water. Both Kondakis et al. and Bouchard et al. correlated high hair manganese to neurological test results. The Kondakis study did not find significant differences in blood manganese between the high and low exposed groups. It is, however, possible that the syringes contained manganese which may have contaminated the samples.

There are however, several difficulties with the use of hair as a biomarker since hair texture and colour, detergent use, hair growth rate as well as distance from the scalp that the hair is taken may affect the manganese concentration making dietary intake or status only one factor contributing to this index (Lönnerdal, 1994).

4.4 Teeth

According to Ericson et al. (2001) tooth enamel may be a useful measure of the exposure to manganese over a long period of time since enamel crystals provide a longitudinal record of absorption, analogous to levels of pollutants recorded in tree rings. According to Ericson et al. (2006), who used manganese concentrations in tooth enamel to link prenatal exposure to childhood

behaviour, the cusp and root tip of the first molar represent different gestational weeks, why prenatal exposure can be evaluated.

5 Health effects of manganese

Adverse health effects of manganese can occur when the intake is either too high (toxicity) or too low (deficiency) (Aschner et al., 2005). Manganese deficiency is, however, rare since it is a ubiquitous element and is found in many foods (Wasserman et al., 2006). According to Freeland-Graves & Llanes (1994), only two experimentally induced manganese deficiencies in humans have been reported. However, poor manganese status has been reported in patients with osteoporosis, epilepsy, multiple sclerosis, senile cataracts, rheumatoid arthritis, Mseleni joint disease, hydralazine syndrome and exocrine pancreatic insufficiency (Freeland-Graves & Llanes, 1994). No deficiency symptoms have been reported in newborns and infants (Dörner et al., 1989). It is commonly excessive, rather than insufficient intake of manganese that causes health problems.

The main target of manganese toxicity is the central nervous system. A Parkinson-like disorder, manganism, was first noted in 1837 in five pyrolusite mill workers. It has since been reported in hundreds of occupationally exposed workers (Mergler, 1999; WHO, 2004b). Manganism is caused by exposure to very high levels of manganese fumes or dusts. The symptoms include weakness, anorexia, muscle pain, apathy, slow speech, monotonous voice, emotionless facial expression and slow, clumsy movement of the limbs. Contradictory results have been found regarding the irreversibility of manganism symptoms. A study of five Taiwanese smelter workers showed slow progression of the manganism symptoms five years after the initial diagnosis (Huang et al., 1993) while permanent symptoms were present in a young man chronically exposed to manganese via a fungicide two years after the exposure had ceased (Meco et al., 1994). According to WHO (2004b), the effects of manganism are generally irreversible.

While manganese toxicity has been reported from inhalation in occupational settings, it is often regarded as one of the least toxic elements by the oral route.

There are however, several reported cases of toxicity from oral exposure in the non-occupational environment. Two studies describe cases where symptoms of manganism occurred as a result of ingestion of mineral supplements. One individual took large supplements for several years, while the other displayed symptoms similar to Parkinson disease nine months after a four week episode of ingesting in total 10 g potassium permanganate (WHO, 2004b; Holzgraefe et al., 1986).

Cases of toxicity have also been reported from elevated levels in drinking water. An epidemiological study in Greece of long-term exposure (more than two years) to naturally occuring manganese in drinking water found correlations between manganese intake and neurological effects in elderly persons (Kondakis et al., 1989). Two groups of persons aged above fifty years of age had been exposed to manganese concentration of 80-250 µg/L and 1800-2300 µg/L, respectively. The control area had a drinking water concentration of 4-15 µg Mn/L. The authors observed a progressive increase in neurological signs of manganese poisoning as well as increased hair manganese with progressively higher manganese levels in drinking water. However, no data was collected on exposure from additional sources such as food and dust., but the authors reported similar food sources for the different groups. Contradictory to the findings of the Greek study, a German study found no correlations between manganese drinking water concentrations above 300 µg/L and neurological effects in elderly people (Vieregge et al., 1995). Like in the Greek study, no data on additional sources of manganese were investigated, and the range of manganese in the drinking water was very wide (WHO, 2004b).

5.1 Health effects in children

At environmental exposures to manganese, few cases of negative health effects have been reported in adults. Instead, children are more vulnerable to adverse health effects of manganese because of their sensitive nervous system, their low bile excretion, high gastrointestinal absorption and homogenous diet. Exposure to excessive manganese has been linked with neurological disorders in children, mainly in the form of behavioral effects. Regardless of exposure route, the primary site of manganese neurotoxicity seems to be the basal ganglia. This has been observed in humans, monkeys, rabbits as well as rats

(Dobson et al., 2004). Several studies have found correlations between behavioural disorders and manganese exposure. Collipp et al. (1983) found hair manganese concentrations in older children with ADHD at twice the level found in a control group. The older children were, however, not followed from birth, so it was not possible to evaluate if the higher hair manganese concentration was a result of excessive manganese intake in younger years.

Ericson et al. (2006) reported correlations between 11 and 13 year old children's behaviour and manganese concentrations in tooth enamel representative of exposure at the 20th and the 62-64th gestational weeks. However, only twenty-seven children were included in the study. Prenatal exposure at the 20th week was reportedly significantly correlated with specific behavioural outcomes; children with higher prenatal manganese exposure were more impulsive, inattentive, aggressive, defiant, disobedient, destructive and hyperactive. The children did not, however, perform lower scores on a standardized test of cognitive ability and achievement. Takser et al. (2003) found supporting results as they followed 100 Parisian children from birth to preschool years. Negative relationships were found between cord blood manganese levels and psychomotor sub-scales of attention, non-verbal memory and hand skills at 3 years of age. These results were observed also after adjustment for the child's gender and mother's educational level. However, no significant relationships were found between cord blood manganese concentration and general psychomotor indices at this age, or for the other age groups investigated (9 months and 6 years). No significant results were obtained for psychomotor sub-scales at the age of six, which may be explained by other socio-cultural factors.

Golub et al. (2005) investigated possible neurobehavioural effects in rhesus monkey infants fed cow's milk formula (50 µg Mn/L), soy formula (300 µg Mn/L) or soy formula with added manganese (1000 µg Mn/L). They observed that both of the two groups of eight infants receiving soy formula were engaged in less play and more passive affiliation than the control group receiving cow's milk. The formula groups also had shorter wake cycles and shorter periods of daytime inactivity. The authors mainly found differences between the groups receiving the different types of formula, i.e. milk and soy, while only a few effects were attributed to differences in manganese concentration. The authors explain this lack of dose effect by the larger

difference in manganese concentrations between the cow's milk formula and the soy milk formula (6-fold), than between the two soy formulas (3-fold).

A pilot study of thirty-one 11-13 year olds reported that manganese and arsenic levels of hair samples were significantly inversely related to general intelligence scores. The main effect for each metal was observed in children with low scores and with both hair manganese and arsenic concentrations above the median. No correlation was found for hair cadmium and IQ. In particular, inverse relationships were found between hair arsenic and manganese concentrations and verbal IQ scores as well as memory for stories and a word list. In contrast to other studies, no relationships were found between hair metal concentrations and behaviour. It should be noted that the limited sample size complicates adjustment for confounding factors. Moreover, the studied children live in a region with a long history of lead mining, but their past lead exposure was not evaluated. Out of the eighty children that were initially approached, around 40% participated after consent from parents. It is thus possible that mostly parents who were already concerned with possible effects from pollution in the area chose to participate (Wright et al., 2006).

Relationships have thus been found for different biomarkers, different cognitive tests and different sources of exposure. Although no single study is entirely convincing as to the level of concern for toxicity in infants and young children, the number of indicative studies makes it reasonable to assume that children are at considerably higher risk for toxicity than older individuals. It is at present not clear whether manganese exposure affects both younger and older children, or if negative symptoms of older children are an effect of infant exposure. In all of the studies involving older children, they could well have been exposed since early life, which does not exclude induction of a neurotoxic effect already during brain development.

5.1.1 Health effects from manganese in drinking water

Several studies have reported relationships between manganese intake from drinking water and behaviour. Woolf et al. (2002) reported of a 10 year old boy who consumed drinking water with a manganese concentration of 1200 μg/L in his home for a period of five years. He had elevated whole blood, urine and hair manganese concentrations and showed below average performance on

memory tests but normal results on IQ and cognitive tests. His brother also had elevated hair manganese concentrations but normal blood levels.

He et al. (1994) and Zhang et al. (1995) reported decreased neurobehavioral performance in 92 matched-pair students aged 11-13 who were exposed to excess manganese through ingestion of contaminated water and from wheat fertilized with sewage water (WHO, 2004b). The average manganese concentration of the drinking water of the exposed group of children was 240-350 µg/L compared to 30-40 µg/L in the control group. The concentration of hair manganese was significantly higher in the exposed group than in the control group (1 and 1.3 mg/g, respectively). The exposed group also had significantly lower scores on several neurobehavioural tests (He et al., 1994). A follow-up study of the same population reported that the higher manganese exposure was associated with lower mathematics and language scores compared to the control group. The latter study also reported higher blood manganese levels (Zhang et al., 1995).

Wasserman et al. (2006) investigated the relationship between intellectual functioning in 142 ten-year old children and well water manganese in Bangladesh. They found an association between manganese exposure and neurotoxic effects and a dose-response association between water manganese concentrations and test scores of performance as well as verbal ability. The water manganese concentration averaged 800 µg/L and ranged from 4 to 4000 µg/L. The children were divided into four groups with different manganese exposure levels (<200 µg/L, 200-499 µg/L, 500-999 µg/L, ≥1000 µg/L). A significant difference in full scale, performance and verbal test results were found between the low exposure and high exposure groups, both before and after adjustment for other factors, including water arsenic concentration. Decreasing test results were found for increasing manganese concentrations for all groups, but the associations were not significant.

Bouchard et al. (2007) found significant associations between manganese concentration in tap water from Quebec, Canada and hyperactivity as well as oppositional behaviour in children aged 6-15. Forty-six children participated in the study of which 28 lived in houses with elevated manganese levels in their tap water (average 600 µg/L). The tap water in the houses of the remaining children had average manganese concentrations of 160 µg/L. The children in the first group had elevated hair manganese concentrations (mean 6.2±4.7 µg/g

compared to 3.3±3.0 μg/g). The strong correlation between manganese in water and in hair suggested that tap water was a significant manganese exposure route. The hair manganese concentrations were significantly associated with test scores of oppositional behaviour and hyperactivity.

6 Water treatment

Manganese can be removed from water in much the same way as iron; by aeration settling and filtrations. Oxidation can be facilitated by adding oxidant such as chlorine, hypochlorite (bleach) or permanganate. Manganese can also be removed via ion exchange using exchange resins or zeolite. Domestic water softeners can also be used for manganese removal, but they are less efficient when concentrations are high (several mg/L). Removal can also be achieved through adsorption to suitable materials, such as clay. Polyphosphates are often used in water distribution systems to prevent the build up of manganese oxide deposits. They complex manganese and prevent oxidation and precipitation, although the complexes can break down at high temperatures (e.g. in boilers). The technique is useful at moderate concentrations of dissolved iron and manganese but is less effective where concentrations of the two reach in excess of 1,000 μg/L (BGS & WaterAid, 2003).

7 Present recommendations for manganese intake in infants

7.1 Scientific background to the NOAEL and LOAEL

The reasoning behind the setting of the present recommendations for manganese intake, including upper limits (UL), adequate daily intakes (ADI), maximum value for infant formula and guideline value for drinking water, all include reference to a 'No Observed Adverse Effect Level' (NOAEL) and/or a 'Lowest Observed Adverse Effect Level' (LOAEL). The following sections provide the background to these values.

7.1.1 No Observed Adverse Effect Level

The 'No Observed Adverse Effect Level', NOAEL is used in setting tolerable upper limits for manganese intake, adequate daily intake values and the health based guideline value for drinking water.

The use of 11 mg as a NOAEL in the background document for the WHO drinking water guidelines (WHO 2004), is based on a review by Greger (1999) and on a report on dietary reference values for manganese published by the Institute of Medicine in 2002 (IOM, 2002), which also refers to Greger (1999). However, the article by Greger (1999) did not focus on adverse effect levels of manganese intake but rather on potential biomarkers of manganese in human nutrition and toxicology. Greger refers to Gibson (1994) and Freeland-Graves (1994) in her presentation of manganese intakes from typical Western-type and vegetarian diets (0.7-10.9 mg Mn/day). However, no mentioning of these values is made in Gibson (1994), whose article is a review on trace elements in vegetarian and omnivorous diets with a concern for deficiencies in vegetarian diets. The Freeland-Graves (1994) article is also a review paper published in a book on risk assessment of essential elements by the International Life Science Institute. Intake values of 0.7-10.8 mg Mn/day were observed for Canadian women in a study carried out by Gibson and Scythes in 1982 and were in the Freeland-Graves (1994) review presented in a table of daily manganese intakes compiled from several studies.

The values used for setting the NOAEL thus originate from one study, where 100 Canadian women aged 30 ± 6.1 years were asked to complete dietary protocols of all consumed foods and beverages (including drinking water) in their own homes for three consecutive weekdays. The calculated daily manganese intake ranged from 0.7 to 10.8 mg, where 90% of the women ingested less than 5 mg/day, and almost half of the women (40%) ingested less than 2.5 mg manganese per day. The NOAEL of 11 mg/day is thus based on calculated daily intakes of manganese, and not on actual measurements of intakes. No mention is made of the subjects' health status. In fact, in the study on which the NOAEL is based, only 10% ingested more than 5 mg Mn/day. Similarly, a review of several studies on manganese intakes worldwide reveals that the average daily manganese intake is rather similar regardless of nationality, and although a vegetarian diet may result in higher daily intakes, the intake rarely exceeds 5 mg/day.

7.1.2 Lowest Observed Adverse Effect Level

A value of the Lowest Observed Adverse Effect Level (LOAEL) is used by the European Commission's Scientific Committee on Food (SCF) in setting the maximum levels for manganese in infant formula. According to Velazquez and Du (1994), the LOAEL is based on findings by Kondakis and co-workers who found an association between elevated manganese concentrations in drinking water and neurological symptoms similar to those of Parkinson's disease in people above 50 years of age. The studied persons consumed water with a manganese concentration of 1800-2300 µg/L for more than 10 years (Kondakis et al. 1989). The LOAEL for the Kondakis study was identified as 2 mg/L, corresponding to 0.06 mg/kg/day (Velazquez and Du 1994). On the assumption that a 70 kg person consumes 2 L per day, the U.S. EPA set the drinking water LOAEL at 4.2 mg/day (0.06 mg/kg multiplied by 70 kg) (Greger & Malecki, 1997).

A LOAEL of 15 mg manganese per day is used by the Institute of Medicine (IOM) in setting the upper limit for manganese intake. The LOAEL they use is based on a study by Davis and Greger (1992), where changes in the serum manganese concentrations and the lymphocyte MnSOD activity were statistically significant after 25 and 90 days of manganese supplementation, respectively. Forty-seven women aged around 25 were included in the study, which aimed to investigate the effect of dietary factors on MnSOD activity. Two groups, each with 11 participants, consumed 15 mg amino-acid chelated manganese supplement daily. One of these two groups also consumed 60 mg iron supplement. The manganese chelate was chosen because of previous findings of higher retention rates (80%) in rodents from ingesting manganese in this form rather than in inorganic forms ($MnCl_2$). Because of the rather long period of time required for a response and because of the fairly small response of MnSOD activity to the supplementation, the authors suggest that the manganese intake was approximate to the requirements of the study subjects.

7.2 Tolerable upper intake level

The European Commission's Scientific Committee on Food (SCF) has not set a tolerable upper intake level of manganese. They recognize the neurotoxic effects that have been found from manganese ingestion and inhalation, but chose not to set an upper limit due to the considerable degree of uncertainty

produced by limitations in human data and the absence of NOAELs for critical endpoints from animal studies (EFSA, 2006).

The Food and Nutrition Board of the Institute of Medicine (IOM) stated similar reasons when they chose not to set an upper limit (UL) for manganese intake in infants younger than one year (IOM, 2002). An upper limit (UL) of 11 mg/day was calculated for both males and females above 19 years of age. The value of 11 mg/day corresponds to the estimated NOAEL of manganese. The IOM report also refers to a previous study by Schroeder et al. (1966) where it was calculated that a manganese-rich vegetarian diet could result in daily intakes of 13-20 mg manganese. Since no adverse effects had been noted in these types of diets, the NOAEL was set at 11 mg Mn/day.

The upper limit of 11 mg/day was derived by dividing the NOAEL by an uncertainty factor of 1, motivated by the lack of evidence of human toxicity from lower doses than 11 mg/day. For other age groups, the UL for adults was extrapolated and adjusted on the basis of relative body weight using reference weights. Consequently, for 14-18 year-olds the UL was set at 9 mg Mn/day, for 9-13 year-olds 6 mg/day, 4-8 year-olds 3 mg/day and for 1-3 year-olds an UL of 2 mg Mn/day was set. For infants younger than one year, no upper limit was determined because of the lack of data on adverse effects in this age group and a concern with regard to the lack of ability to handle excess amounts. The IOM report also states that for the non-determinable groups (infants aged 0-6 months and 7-12 months) the source of intake should be from food only to prevent high intake levels (IOM, 2002).

7.3 Adequate Daily Intake

The IOM report on dietary reference values has set the adequate daily intake (ADI) of manganese for infants younger than 7 months at 3 µg. The ADI value is based on the mean manganese intake of infants exclusively fed human milk from well-nourished mothers. The ADI was calculated by multiplying the average milk consumption (0.78 L/day) with the average manganese concentration in human milk (3.5 µg/L) (IOM, 2002). Since the manganese concentration in breast milk varies, the manganese intake for children receiving the upper manganese concentrations through breast milk would ingest 12 µg

manganese daily, i.e. more than the adequate amount set by IOM (WHO, 2004b).

For infants between 7 and 12 months of age the ADI was set at 600 µg/day by the IOM, i.e. markedly higher than that from the younger age group. The reason for this is the additional intake of manganese from complementary foods. The report refers to a study by Gibson and De Wolfe (1980) where the average manganese intakes in 6 and 12-month old infants were 71 and 80 µg/kg, respectively. Using reference weights of 7 and 9 kg, the total average manganese intakes would be 500 and 720 µg/day, respectively. By instead extrapolating the NOAEL for adults (11 mg/day), an average daily manganese intake of 567 µg is attained. By using these two approaches, an adequate daily intake of 600 µg/day was set (IOM, 2002).

7.4 Infant formula and follow-on formula

There are three different recommendations from the Swedish Food and Nutrition Board regarding food stuffs for infants. These are congruent with directives from the European Commission. Recommendation SLVFS 1994:46 (Livsmedelsverket, 2004) regards infant formula and follow-on formula (to be used for infants above the age of four months), and corresponds to the EC Infant Formulae Directive 91/321/EEC (EU, 1991). While no specific value is set for the manganese concentration in infant formula products, follow-on formula should contain at least the same amount as in cow's milk, reduced when required by the differing protein concentration in the product and in cow's milk.

The second recommendation, SLVFS 1997:27, regards processed cereal-based foods and baby foods for infants and young children, aged younger than 12 months and between 12-36 months, respectively. The maximum concentration of manganese for these products is 600 µg/100 kcal (Livsmedelsverket, 1997). This recommendation refers to the EC directive 96/5/EC which has the same maximum values (EU, 1996).

In the third recommendation, SLVFS 2000:15, corresponding to the EC Directive on dietary foods for special medical purposes (1999/21/EC), a minimum value of 50 µg Mn/100 kcal and a maximum value of 200 µg

Mn/100kcal are set for infants younger than 12 months. These values regard foods for infants that are nutritionally complete (Livsmedelsverket, 2000; EU, 1999).

In the report of the European Commission's Scientific Committee on Food (SCF) on the revision of essential requirements of infant formula and follow-on formula, a recommendation of a minimum manganese concentration is made at 1 μg/100 kcal and a maximum concentration of 100 μg/kcal (SCF, 2003), corresponding to 7 and 650 μg Mn/L (Sievers, 2005). These values regard both infant formula and follow-on formula. The minimum concentration is based on the mean manganese concentration in human breast milk (3-4 μg/L corresponding to ~0.5 μg/100 kcal) and regards the lower absorption of manganese from cow's milk and soy-formula. The report recognizes that there is increasing evidence of neurotoxic effects from excessive manganese intake in infants and therefore proposes the maximum value of 100μg/kcal. They also recognize that there is no upper limit set for manganese. The report further motivates the maximum value by stating that it "is below the estimated LOAEL in adults for manganese contents in water (4.2 mg/L)" (SCF, 2003). However, the LOAEL is based on a study by Kondakis et al. (1989) in which a LOAEL of 4.2 mg per day, not per *liter* was set. This miscalculation results in the use of a LOAEL more than two-fold the manganese concentration that was found in the drinking water associated with neurological symptoms in people above 50 years of age. If, instead, the LOAEL based on bodyweight had been used (0.06 mg/kg/day), it would have resulted in 300 μg/day for a 5 kg infant, 420 μg/day for a 7 kg infant and 540 μg/day for a 9 kg infant. On the assumption that a 5 kg infant consumes 0.78 L/day, the LOAEL for manganese in formula would then be 385 μg/L, corresponding to 60 μg/100kcal. It should also be noted that the Kondakis study reported findings on manganese intake in adults aged above 50, and not on infants.

The minimum and maximum values set by the SCF correspond to the manganese values for infant foods set by Life Science Research Office (LSRO). In addition to the motivations for the maximum concentration stated by the SCF, LSRO acknowledges that a highest oral NOAEL has not been established for human or nonhuman primates. Further, they state that the maximum value of 100 μg/100 kcal (corresponding to 650 μg/L) is far beyond the range likely to be encountered in milk-based formulas, and also above the manganese concentration likely to be associated with isolated soy-protein

formulas (Raiten et al., 1998). However, this is not entirely true since, according to the producers, both cow's milk and soy based infant formulas contain between 25-600 µg/L depending on the brand, and analyses have found 30-1,150 µg Mn/L in infant formulas without additional manganese from drinking water (Hozyasz & Ruszczynska 2004; Krachler et al. 2000; Krachler et al. 1998).

The *Codex Alimentarius* Commission has also set standards for infant formulas. The minimum manganese concentration is set at 5 µg/100 kcal while the maximum concentration is left unspecified. (Codex Alimentarius Commission, 1976)

7.5 Drinking water

In the rolling revision of the WHO drinking water guidelines, it was recognized that for certain minerals such as manganese and molybdenum, drinking water may be a significant contributor to the overall intake, particularly for formula-fed infants. The use of drinking water at the guidelines values for various minerals may therefore lead to nutritional requirements being exceeded. (WHO, 2004b)

The WHO has set a health based guideline value of 400 µg Mn/L for drinking water. They recognize that no quantitative data is available indicating toxic levels of manganese in human diet. They have looked at several studies that determine the mean manganese intake in various diets, and because of the homeostatic control that humans maintain for manganese, it is stated that manganese is generally not considered to be very toxic when ingested together with food.

The report refers to two studies, one carried out by the Food and Nutrition Board in cooperation with the Institute of Medicine (IOM, 2002) that have calculated dietary reference intake values for manganese, among other elements. The other study is by Greger (1999) and evaluates potential biomarkers of manganese. The NOAEL set by IOM for dietary manganese intake of adults above 19 years of age has been used to calculate the guideline value for manganese in water. Since it is believed that manganese is more bioavailable when ingested with water than with food, the NOAEL of 11

mg/day is divided by an uncertainty factor of three. Using an adult body weight of 60 kg, a tolerable daily intake (TDI) of 60 μg manganese per kg bodyweight is derived. On the assumption that 20% of the TDI comes from drinking-water since manganese is an essential element, and that an adult consumes 2 liters of water per day, the guideline value for drinking water is set at 400 μg/L (WHO, 2004b).

In Sweden, the quality of the water provided by municipal plants is regulated by legally binding guidelines. The implementation of these guidelines is administered by the Food and Nutrition Board (Livsmedelsverket, 2001). For water provided by municipal services, the manganese value is set at 50 μg/L. This value is in accordance with the EC directive on the quality of water intended for human consumption (98/83/EC) (EU, 1998). About 15% of the Swedish population retrieve their everyday water from private wells. Another 1.2 million people have private wells at their summer homes. There are no legally binding regulations on water quality for private wells. Instead of regulations, guidelines and general advice on water quality are available from the National Board of Health and Welfare (Fagerlind, 2007). The Swedish guideline value for manganese in water from private wells is set at 300 μg/L. This guideline value is technically based rather than health-based, motivated by the risk for build-up in water pipes that may cause staining problems (Socialstyrelsen, 2003). The value for manganese set by the EC is an indicative value and not legally binding, which is why a higher value is allowed for private wells.

8 Risk assessment

The current technically and health based guideline values for manganese in water are likely low enough to protect the health of adolescents and adults. Moreover, there seems to be little evidence of significant manganese transfer from the mother to the fetus through the placenta as well as to the infant through breastmilk, even if the mother is exposed to elevated manganese concentrations in drinking water. However, the many uncertainties regarding bioavailability, absorption, retention, excretion, and health effects of manganese in infants warrant caution regarding the quality of water intended for infants, especially those receiving infant formula.

The current maximum manganese concentration in infant formula set by the European Commission is 650 µg/L (SCF, 2003). Since manganese is an essential element and is less bioavailable in formula than in breast milk, a higher manganese concentration has been considered acceptable in formula in order to provide adequate manganese intake levels to infants. However, it is questionable whether a 100-fold higher manganese concentration in formula is justifiable. Moreover, this health based maximum value for infant formula does not consider additional manganese input from the water with which it has to be mixed. In order for the prepared product not to exceed the set guideline value, the quality of the water is essential. A common manganese concentration of infant formula powder gives around 400 µg/L in the ready-to-eat formula (see Table 2), when no manganese is added from water. Thus, the water used for preparing the formula cannot contain more than 250 µg/L in order not to exceed the maximum formula manganese concentration of 650 µg/L (SCF, 2003). For comparison, the health-based WHO drinking water guideline is 400 µg Mn/L.

In addition to the major uncertainties in the scientific basis for the formula standard of 650 µg/L, an increasing number of studies report relationships between fairly low manganese exposure levels and negative health effects in children. Consequently, this value cannot automatically be applied to drinking water. Relationships have been found for different biomarkers, different cognitive tests and different sources of exposure. Although no single study is entirely convincing as to the level of concern for toxicity in infants, the number of indicative studies makes it reasonable to assume that infants are at

considerably higher risk for toxicity than older individuals. More studies are needed to validate current findings, provide basis for quantitative risk assessment, and to evaluate the suitability of different biomarkers of exposure and adverse effects.

There are several factors that need to be considered in order to establish a health based guideline value for infant water consumption. The bioavailability of manganese in water and formula is apparently lower than that of manganese in breast milk, indicating that manganese concentrations in drinking water exceeding the upper range in breast milk (about 10 µg/L) would be acceptable. At present, most formulas contain a 100-fold higher manganese concentration than breast milk. Studies have also shown that infants consume larger volumes of infant formula than of breast milk, thereby also consuming larger volumes of water, and thus potentially more manganese. Other important considerations are the differing roles of trivalent and divalent manganese with regard to neurotoxicity and the difference in susceptibility to manganese in different age groups.

Out of the approximately 19,000 wells in Sweden that SGU has sampled, around 15% had manganese concentrations above 300 µg/L. If the same relationship (~15%) applies to the remaining private wells in the country, approximately 60,000 wells supplying drinking water to permanent households and just as many supplying temporary households have manganese concentrations in their drinking water that may be unfit for infant consumption.

9 Conclusions and recommendations

The number of questionable assumptions in the development of WHO's current health based guideline value for manganese for drinking water as well as for the maximum manganese concentration in infant formula, in combination with the increasing number of reports on infant neurotoxicity from manganese exposure, certainly warrants a re-evaluation of the current guideline values for manganese in drinking water.

The current Swedish recommendation for private wells of 300 µg Mn/L is likely low enough to protect the health of adults and adolescents. It is also probable that this level is low enough to protect the unborn fetus and breastfed infant from maternal exposure, since transport from the mother appears to be limited. Moreover, this value is well below the LOAEL of around 2000 µg/L at which negative symptoms were reported in elders (Kondakis et al. 1989). However, it is hasty to draw conclusions based upon a single study, which emphasizes the need for further research within the area of especially elder's exposure to manganese and any connections with Parkinson-like symptoms. There is also a need for more research on transport from the mother to the fetus and child as well as the potential effects of manganese accumulation in placenta.

In contrast to studies on elder's exposure to manganese, there are several studies indicating that excessive exposure to manganese may have negative effects on infant and child health. It is at present not clear whether manganese exposure affects both younger and older children, or if negative symptoms of older children are an effect of fetal or infant exposure. In any case, the current recommendation of 300 µg/L is not likely to exert a significant health risk to children consuming normal quantities of water, as this amount adds up to only 15% of a child's average daily manganese intake (~2 mg/day), assuming that one liter per day is consumed.

The main risk group for exposure to excessive manganese through drinking water is formula fed infants. As has been stated above, most formulas alone contain significant amounts of manganese, even prior to mixing with water, why water quality is essential to the well-being of these infants. At present, if common formula (manganese concentration ~400µg/L) is mixed with water

containing 300 µg Mn/L, the maximum manganese level for formula set by the EU is exceeded. In addition, the scientific evidence for the protection of infant health at this maximum value is questionable. It is therefore advisable that both the formula and the water used for mixing the formula contain as little manganese as possible, in order not to significantly exceed the normal manganese concentration found in human breast milk.

The maximum manganese level of 300 µg/L for drinking water is a recommendation and not legally binding, and it is hence up to each household to be responsible for its water quality. In order to ensure that infants are not exposed to excessive manganese levels, it is thus necessary to inform parents about the importance of water quality with regard to their babies' health. Because of the International Code of Marketing of Breast Milk Substitutes, distribution of information about infant formula is restricted to government health officials. This is thus likely an appropriate and efficient information channel to parents. It is therefore recommended that health officials inform parents of infants receiving formula about the risks of manganese exposure to the youngest and provide advice on how to avoid any potential elevated manganese exposure.

As a substitute to tap water, formula can be mixed with bottled water. There are four types of bottled water on the Swedish market: mineral water, spring water, bottled drinking water and table water. While the maximum manganese concentrations of the two former types is set at 500 µg/L, the two latter types have the same restrictions as water from public water services; 50 µg/L, which can then be used for mixing formula, if the household drinking water has elevated manganese concentrations. Moreover, filters can be used to remove manganese from drinking water.

In conclusion, there are still several uncertainties about the lowest level for adverse health effects from manganese exposure, especially in infants and children, but also in elders and in the unborn child. Further research is needed to understand the causal relationship between manganese exposure and children's health, and to enable an improved risk assessment. More specifically, research on the difference in manganese bioavailability between breast milk, formula and water is needed as well as a greater understanding of manganese absorption and retention in different age groups. Moreover, there is

an urgent need for a useful biomarker in order to assess a dose-response relationship.

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